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Conjugative Transposition of Tn*916*-Related Elements from *Enterococcus faecalis* to *Escherichia coli* and *Pseudomonas fluorescens*

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We studied the ability of transposons Tn*916*, Tn*1545*, and Tn*916*-Km, a Tn*916* derivative expressing kanamycin resistance, to be conjugatively transferred from *Enterococcus faecalis* to various gram-negative bacteria. Our results demonstrate that these types of elements can carry out conjugative transposition from the chromosome of *E. faecalis* to those of *Escherichia coli* and *Pseudomonas fluorescens* and that the accomplishment of this event depends on the donor potential of the *E. faecalis* transposon delivery strain. Since the *tet*(*M*) gene does not confer a selectable level of tetracycline resistance to gram-negative bacteria such as *E. coli*, the presence of another marker(s) readily expressed in these recipients is required for the detection of this type of transfer. Conjugal transfer of Tn*916*-Km from *E. faecalis* to *E. coli* is not restricted by the *Eco*K restriction system, nor does it depend on the presence of functional homologous recombination system and integration host factor proteins in the recipient bacteria.

The 16.4-kb transposon Tn*916*, detected in the chromosome of *Enterococcus faecalis* DS16 (11), and the 25.3-kb transposon Tn*1545*, originally found in the multiply antibiotic-resistant *Streptococcus pneumoniae* BM4200 (9), are the prototypes of a family of conjugative elements which are widespread in gram-positive cocci and bacilli (2, 24, 25). Transposable elements belonging to this family are large DNA segments (≥16 kb), and all contain the tetracycline resistance determinant *tet*(*M*) alone (e.g., Tn*916*) or associated with other resistance genes (e.g., Tn*1545*). Transposition of these elements proceeds by excision to a free, nonreplicative, covalently closed circular intermediate that is a substrate for integration (8, 29). Conjugal transfer of Tn*916* and related elements can be viewed as a transposition event involving donor and recipient replicons that happen to be present in different cells and was therefore referred to as conjugative transposition. This event involves circularization of the element in the donor and transfer of the nonreplicative intermediate to the recipient, where it integrates (31). Thus, in addition to their transfer functions for which no data are currently available, transfer of this class of elements requires transposon-encoded excision and integration functions (22, 23, 31). There is circumstantial evidence that conjugal transfer of Tn*925*, an element closely related to Tn*916*, proceeds through cell fusion (32), but this hypothesis is controversial (10, 31).

Conjugative transposons Tn*916* and Tn*1545* are powerful tools for mutagenesis in gram-positive bacteria of medical or industrial importance because of their broad host range (7, 27). The frequency of conjugal transfer of these elements in matings on solid media is usually between 10^{-6} and 10^{-8} per donor, depending on the donor and the recipient. Conjugal transfer of Tn*916* from *Bacillus subtilis* to the gram-negative *Thermus aquaticus* (30) and from *E. faecalis* to the gram-negative *Butyrivibrio fibrisolvens* (14) has been reported. However, although these recipients appear as gram-negative cells when stained by conventional procedures, partial sequence analysis of their 5S or 16S rRNA revealed that they are of gram-positive phylogenetic origin (14, 30). The frequencies of transfer of Tn*916* from *E. faecalis* to *Alcaligenes eutrophus*, *Citrobacter freundii*, and *Escherichia coli* have been reported to range from 10^{-6} to 10^{-8} (3). In these experiments, however, Tn*916* was carried by a self-transferable plasmid in the donor bacterium, and it is therefore not possible to determine if the transfer observed was mediated by the *tra* functions of the transposon, by those of the bearing replicon which could have played the role of a transposon delivery vector, or by both *tra* systems, which could have complementary activities. The experiments described in this article demonstrate that elements belonging to the Tn*916* transposon family can carry out conjugative transposition from the *E. faecalis* chromosome to *E. coli* and *Pseudomonas fluorescens.*

MATERIALS AND METHODS

Bacterial strains and growth conditions. The main characteristics of the bacterial strains, plasmids, and transposons used in this study are listed in Table 1. The plasmid-free *E. faecalis* BM4110 and JH2-2 are devoid of sequences related to either Tn*916* or Tn*3701* (19), which are the prototypes of the two classes of conjugative elements described so far for enterococci and streptococci. Moreover, these strains were unable to transfer nonconjugative plasmids by filter matings, a finding which indicates that they did not encode chromosome-borne mobilization functions (33).

DNA manipulations, Southern blotting, and PCR amplification. Total and plasmid DNAs were extracted from *E. coli* as described elsewhere (26). Total DNAs from *B. subtilis* and *E. faecalis* were purified as previously described (31). Restriction endonuclease-generated DNA fragments were separated by electrophoresis on 0.8% agarose gels and transferred to Nytran membranes (26). Prehybridization and hybridization under stringent conditions were carried out as described previously (25). DNA fragments were labeled with [α-^{32}P]dCTP (Amersham S. A.) by nick translation (26). Amplification was performed in a final volume of 100 μl containing 50 ng of genomic DNA, 0.1 μM each primer, 200 μM each deoxynucleoside triphosphate, and 2 U of *Taq* DNA polymerase in a 1× amplification buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$). The PCR mixture was submitted to a denaturation step (3 min at 95°C), followed by 35 cycles of amplification (1 min of annealing at 65°C, 90 s of elongation at 72°C, and 30 s of denaturation at 95°C).

Genetic techniques. Filter matings between gram-positive bacteria were as previously described (9). Filter matings between *E. faecalis* and gram-negative bacteria were carried out as follows. Overnight cultures of donor and recipient

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TABLE 1. Bacterial strains and transposons used in this study

Strain[a], plasmid, or transposon	Relevant properties[b]	Reference or origin
Strains		
E. faecalis		
BM4110[c]	Str^r	9
JH2-2[c]	Rif^r Fus^r	15
EFCP1	BM4110::Tn*916*	This study
EFCP2	JH2-2::Tn*916*-Km	This study
EFCP3	JH2-2::Tn*916*-Km	This study
EFCP4	JH2-2::Tn*1545*	This study
EFCP5	EFCP1::Tn*1545*	This study
EFCP6	EFCP2::Tn*1545*	This study
EFCP7	EFCP2::Tn*916*	This study
B. subtilis		
W168::Tn*916*	*trp* Str^r Tc^r	31
W168::Tn*916*-Km	*trp* Str^r Km^r	This study
E. coli		
JM83	F$^-$ *ara* Δ(*lac-proAB*) *rpsL* (Str^r) ϕ80 dΔ*lacZ*M15	21
TB1	Same as JM83 but *hsdR* (r_{k-} m_{k+})	17
HB101	F$^-$ Δ(*gpt-proA*)*62 leu supE44 ara14 galK2 lacY1* Δ(*mcrC-mrr*) *rpsL* (Str^r) *xyl-5 mtl-1 recA13*	4
RR1	Same as HB101 but RecA$^+$	26
C600	F$^-$ e14$^-$ (McrA$^-$) *thr-1 leuB6 thi-1 lacY1 supE44 rfbD1 fhuA21*	M. Chandler
MC420	Same as C600 but Δ(*hip*)*3*::*cam*	M. Chandler
C. fetus 5396	Wild type	36
P. fluorescens BM2687-2	Cured derivative of the wild-type strain BM2687	18
Plasmids		
pTCV1::Tn*916*	Sm^r/Sp^r Tc^r; a pGB2-derivative carrying Tn*916* integrated in a synthetic 32-mer *Bam*HI oligonucleotide	J. Celli and P. Trieu-Cuot (35)
pTCR4	Ap^r; pUC18 with a 2-kb *Hin*dIII-*Kpn*I fragment intragenic to *tet*(*M*)	20; this study
pTCR5	Ap^r Km^r; pTCR4 with a 1.5-kb *Cla*I fragment carrying *aph*(*3′*)-*IIIa*	34; this study
pTCR6	Ap^r; pUC18 with a 1.5-kb *Eco*RI fragment carrying *int-Tn*	C. Poyart and P. Trieu-Cuot
Transposons		
Tn*916*	Tc^r [*tet*(*M*)] Tra$^+$	12
Tn*916*-Km	Km^r [*aph*(*3′*)-*IIIa*] Tra$^+$	This study
Tn*1545*	Km^r [*aph*(*3′*)-*IIIa*] Em^r (*ermB*) Tc^r [*tet*(*M*)] Tra$^+$	9

[a] Bacteria were grown in brain heart infusion broth or agar.
[b] Em^r, Fus^r, Km^r, Rif^r, Sm^r (or Str^r), Sp^r, and Tc^r, resistance to erythromycin, fusidic acid, kanamycin, rifampin, streptomycin, spectinomycin, and tetracycline, respectively.
[c] Spontaneous mutant of JH2.

cells were diluted 1:20 in fresh brain heart infusion broth without antibiotics. Cells were grown for approximately 5 h, and donor and recipient were mixed in a 20:1 ratio. Aliquots of 200 μl of the mating mixture were spread on 0.45-μm-pore-size nitrocellulose membrane filters (Millipore Corp.) placed on brain heart infusion agar containing DNase I (100 U/ml). After 18 h of incubation at 37°C, the cells were suspended in 2 ml of broth and plated on agar containing appropriate antibiotics to select transconjugants. Gram-negative transconjugants were identified by morphological and biochemical tests (e.g., Gram staining and catalase reaction to discriminate *E. faecalis* from *E. coli* and *P. fluorescens*). Recombinant plasmid DNAs were introduced by transformation into *E. coli* (26) and *B. subtilis* (1).

Construction of Tn*916*-Km. The 2-kb *Hin*dIII-*Kpn*I fragment intragenic to *tet*(*M*) (20) was inserted into pUC18 digested with *Hin*dIII and *Kpn*I to give pTCR4. This plasmid has a single *Cla*I site which is in the *tet*(*M*) gene. The 1.5-kb *Cla*I DNA fragment bearing the kanamycin resistance gene *aph*(*3′*)-*IIIa* expressed in both gram-positive and gram-negative bacteria (34) was inserted in this site. A recombinant plasmid designated pTCR5, in which *aph*(*3′*)-*IIIa* and the disrupted *tet*(*M*) gene have the same polarity of transcription, was used to transform competent cells of *B. subtilis* W 168::Tn*916*. Transformants were selected on brain heart infusion agar containing 50 μg of kanamycin per ml. Twelve transformants were selected, purified, and tested for antibiotic resistance by agar disk diffusion. Their genomic DNAs were isolated, digested with *Hin*cII, and analyzed by Southern hybridization using ^{32}P-labeled pTCV1::Tn*916* as a probe to detect sequences related to Tn*916*. As expected, all the clones were resistant to kanamycin but susceptible to tetracycline. Their hybridization patterns were indistinguishable and showed, compared with the parental element, a size increase of about 1.5 kb (data not shown and Fig. 2, lanes 1 and 8) of the 4.8-kb *Hin*cII fragment which contains *tet*(*M*) (5). One of these transformants was selected for further studies, and the absence of pUC18 sequences in this strain was confirmed by dot blot hybridization (data not shown). A hypothetical model for the construction of this Tn*916*-derivative conferring resistance to kanamycin, and hence designated Tn*916*-Km, is shown in Fig. 1. Tn*916*-Km was subsequently transferred by conjugation into *E. faecalis* JH2-2.

RESULTS AND DISCUSSION

Screening of transposon delivery *E. faecalis* strains having high donor potential. As previously reported (12), we observed that the frequency of conjugative transposition of Tn*916* between isogenic strains of *E. faecalis* may vary significantly between different Tn*916*-containing donors. The differences in Tn*916* donor potential are thought to reflect the chromosomal

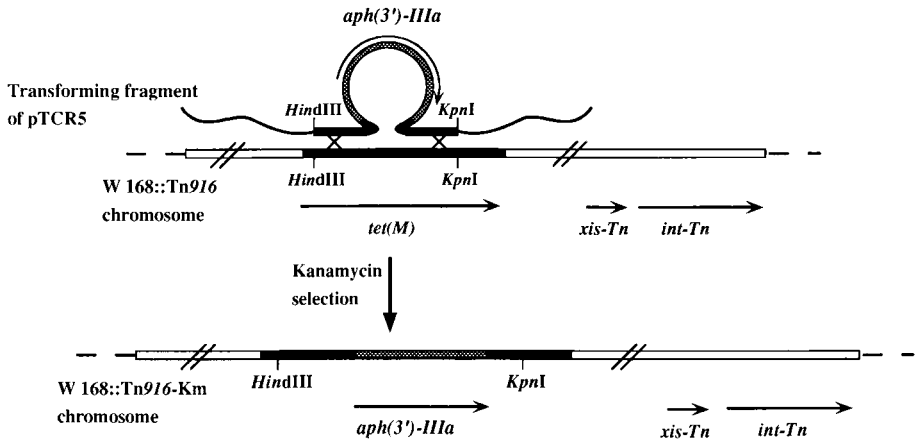

FIG. 1. Construction of *B. subtilis* W 168::Tn*916*-Km. The selected transformant resulted from a double crossover event involving homologous sequences present in the chromosome of W 168::Tn*916* and in pTCR5 (solid boxes). DNAs from Tn*916*, *tet*(*M*), and *aph*(*3′*)-*IIIa* are represented by open, solid, and shaded segments, respectively. Chromosomal DNA and the pUC18 sequences in pTCR5 are depicted by dashed and solid lines, respectively. *aph*(*3′*)-*IIIa*, gene encoding 3′-aminoglycoside phosphotransferase; *tet*(*M*), gene encoding TET(M) protein; *xis-Tn* and *int-Tn*, genes encoding the excisionase and integrase of Tn*916*, respectively. Horizontal arrows, direction of transcription.

location of the transposon in the donor strains (12). Recent experiments suggest that the 6-bp transposon-chromosome junction sequences play a frequency-determining role in conjugative transposition of Tn*916* in *E. faecalis* (16). Therefore, to screen efficient transposon delivery donors, Tn*916*, Tn*916*-Km, and Tn*1545* were transferred from one *E. faecalis* donor (BM4110 or JH2-2) to the suitably marked isogenic recipient. The frequency of transfer of each transposon was then determined. Of the transconjugants produced, six were evaluated by having them act as donors in an ensuing round of transfer. This was performed 10 times, implying that 60 transconjugants were tested for donor potential for each transposon. *E. faecalis* EFCP1, EFCP2, and EFCP4 were the most efficient strains selected by using this procedure for delivery of Tn*916*, Tn*916*-Km, and Tn*1545*, respectively (Table 2). We evaluated the number of copies of transposons present in these strains by determining the number of transposon-chromosome junction fragments. Chromosomal DNAs were prepared, digested with *Hin*cII, and probed with ^{32}P-labeled Tn*916* and *int-Tn* purified from pTCV1::Tn*916* and pTCR6, respectively. For a

TABLE 2. Conjugative transposition of Tn*916*, Tn*916*-Km, and Tn*1545* from *E. faecalis* to various bacterial species

E. faecalis donor[a]	Recipient	Antibiotic selection[b]	Transfer frequency (avg ± SD)[c]
EFCP1	*E. faecalis* JH2-2	RIF, FUS, TC	$(2.3 \pm 1.8) \times 10^{-5}$
EFCP1	*E. coli* TB1	VAN, TC	$<10^{-9}$
EFCP1	*E. coli* RR1	VAN, TC	$<10^{-9}$
EFCP1	*C. fetus* 5396	VAN, TC	$<10^{-9}$
EFCP1	*P. fluorescens* BM2687-2	VAN, TC	$<10^{-9}$
EFCP2	*E. faecalis* BM4110	SM, KM	$(4.1 \pm 1.3) \times 10^{-4}$
EFCP2	*E. coli* TB1	VAN, KM	$(8.3 \pm 1.7) \times 10^{-9}$
EFCP2	*E. coli* JM83	VAN, KM	$(6.7 \pm 1.9) \times 10^{-9}$
EFCP2	*E. coli* RR1	VAN, KM	$(3.5 \pm 1.7) \times 10^{-9}$
EFCP2	*E. coli* HB101	VAN, KM	$(4.2 \pm 2.1) \times 10^{-9}$
EFCP2	*E. coli* C600	VAN, KM	$(1.5 \pm 1.6) \times 10^{-9}$
EFCP2	*E. coli* MC420	VAN, KM	$(1.3 \pm 1.4) \times 10^{-9}$
EFCP2	*C. fetus* 5396	VAN, KM	$<10^{-9}$
EFCP2	*P. fluorescens* BM2687-2	VAN, KM	$(1.8 \pm 1.1) \times 10^{-9}$
EFCP3	*E. faecalis* BM4110	SM, KM	$(7.2 \pm 1.5) \times 10^{-6}$
EFCP3	*E. coli* TB1	VAN, KM	$<10^{-9}$
EFCP4	*E. faecalis* BM4110	SM, KM	$(3 \pm 0.9) \times 10^{-6}$
EFCP4	*E. coli* TB1	VAN, KM	$<10^{-9}$
EFCP4	*E. coli* RR1	VAN, KM	$<10^{-9}$
EFCP4	*C. fetus* 5396	VAN, KM	$<10^{-9}$
EFCP4	*P. fluorescens* BM2687-2	VAN, KM	$<10^{-9}$

[a] EFCP1, BM4110::Tn*916*; EFCP2, JH2-2::Tn*916*-Km; EFCP3, JH2-2::Tn*916*-Km; EFCP4, JH2-2::Tn*1545*.

[b] Antibiotics were used at the following concentrations (in micrograms per milliliter) for gram-negative bacteria: kanamycin (KM), 75; streptomycin (SM), 100; tetracycline (TC), 8; vancomycin (VAN), 20. For *E. faecalis* the concentrations were as follows: fusidic acid (FUS), 20; KM, 1,000; rifampin (RIF), 40; SM, 1,000; TC, 8.

[c] Number of transconjugants per donor CFU after mating. The data are means for three independent experiments.

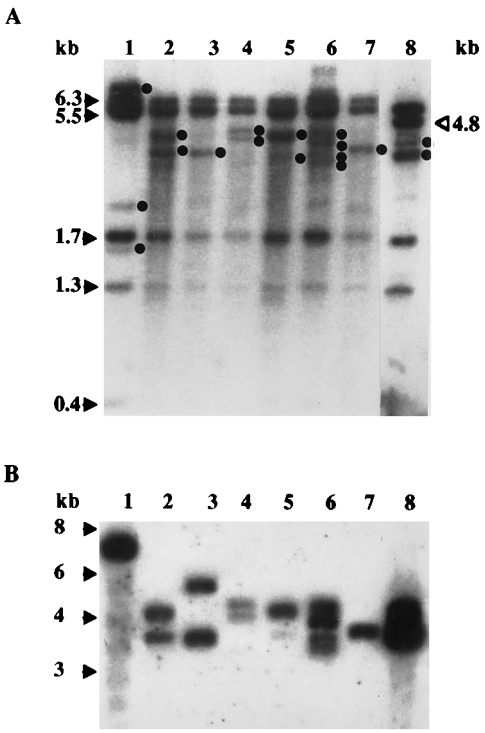

FIG. 2. Insertion of Tn*916*-Km in the chromosomes of *E. coli* transconjugants. Bacterial DNAs from *E. faecalis* EFCP2 (JH2-2::Tn*916*-Km) (lane 1), six selected transconjugants of *E. coli* TB1::Tn*916*-Km originating from the same mating (lanes 2 to 7), and *E. faecalis* EFCP1 (BM4110::Tn*916*) (lane 8) were purified, digested with *Hin*cII, resolved by agarose gel (0.8%) electrophoresis, transferred onto a Nytran membrane, and hybridized with ^{32}P-labeled Tn*916* (A) or *int-Tn* (B) to detect sequences related to Tn*916* and to identify the transposon-chromosome junction fragment(s) carrying *int-Tn*, respectively. The molecular sizes of *Hin*cII DNA fragments internal to Tn*916*-Km are indicated on the left in panel A. The *Hin*cII fragment of Tn*916* carrying *tet*(*M*) is indicated on the right. Dots, transposon-chromosome junction fragments.

given insertion, the Tn*916* DNA probe hybridizes with the five *Hin*cII internal fragments and the two transposon-chromosome junction fragments, whereas *int-Tn* hybridizes with only one junction fragment (Fig. 2). In the case of EFCP2, three junction fragments were characterized on the basis of their size variability, and it was therefore assumed that this strain harbored a minimum of two copies of Tn*916*-Km (Fig. 2, lanes 1). A similar analysis revealed that a minimum of three copies of Tn*916* and two copies of Tn*1545* were present in *E. faecalis* EFCP1 and EFCP4, respectively (data not shown). These results confirmed that the donor potential of a given strain is not related to the number of copies of chromosome-borne transposons (7). *E. faecalis* EFCP3, which delivered Tn*916*-Km 50 times less efficiently than EFCP2 (Table 2), was also retained for further studies.

Conjugative transposition of Tn*916*-Km, but not Tn*916*, from *E. faecalis* to gram-negative bacteria. Conjugative transposition of Tn*916* from *E. faecalis* EFCP1 to various strains of *E. coli*, *Campylobacter fetus*, or *P. fluorescens* was not detected by filter mating (Table 2). The *tet*(*M*) genes carried by Tn*916* and by Tn*1545* do not confer detectable resistance to tetracycline when they are in the *E. coli* chromosome (6, 9, 13). Thus, the apparent inability to transfer Tn*916* by conjugation from *E. faecalis* to gram-negative recipients might have been due to the failure of the transposon-borne *tet*(*M*) gene to express a sufficient level of tetracycline resistance in these hosts. Indeed,

TABLE 3. Conjugative transposition of Tn*916* from *E. faecalis*[a] to *E. coli* by *trans* activation

Recipient and antibiotic selection[b]	No. of transconjugants/donor (avg ± SD)[c]	Transposon transfer selection	% Transconjugants that also received Tn*916*
E. faecalis BM4110			
SM, TC	$(8 \pm 1.6) \times 10^{-5}$	Tn*916*	
SM, KM	$(1.3 \pm 1.2) \times 10^{-4}$	Tn*916*-Km	50[d]
E. coli TB1			
VAN, TC	$<10^{-9}$	Tn*916*	
VAN, KM	$(5.7 \pm 1.6) \times 10^{-9}$	Tn*916*-Km	40[e]

[a] EFCP7 (EFCP2::Tn*916*).
[b] Antibiotics were used at the concentrations indicated in Table 2, footnote *b*. SM, streptomycin; TC, tetracycline; KM, kanamycin; VAN, vancomycin.
[c] The data are means for three independent matings.
[d] Percent transconjugants resistant to tetracycline.
[e] Percent transconjugants that harbored Tn*916*, determined by PCR (Fig. 3).

conjugative transposition of Tn*916*-Km from EFCP2 to various strains of *E. coli* and *P. fluorescens*, but not to *C. fetus*, was detected at low frequencies (Table 2). The presence of Tn*916*-Km in these transconjugants was confirmed by Southern analysis of their *Hin*cII-digested chromosomal DNAs using as probes ^{32}P-labeled Tn*916* and *int-Tn* purified from pTCV1::Tn*916* and pTCR6, respectively. This analysis revealed that most transconjugants harbored multiple copies of Tn*916*-Km inserted at various locations in the host chromosome, as deduced by the number and the size variability of the transposon-chromosome junction fragments, and that no detectable rearrangements had occurred within the transposon during the conjugative transposition process (Fig. 2 shows part of this analysis). Tn*916*-Km transferred at similar frequencies from EFCP2 to various pairs of *E. coli* strains: (i) TB1-JM83 (indicating that the transfer of this element is not restricted by the *Eco*K restriction system) (Tables 1 and 2), and (ii) RR1-HB101 and C600-MC420 (suggesting that conjugative transposition does not depend on the presence of functional homologous recombination system (RecA) or integration host factor proteins in the recipient bacteria, respectively) (Tables 1 and 2). No conjugal transfer ($<10^{-9}$ per donor) of Tn*916*-Km from any of these *E. coli* transconjugants to *E. coli* or *E. faecalis* recipients was detected (data not shown). Interestingly, transfer of Tn*916*-Km from *E. faecalis* EFCP3 to *E. coli* TB1 was never obtained (Table 2). This result indicates that the ability of Tn*916*-Km to undergo conjugative transposition from *E. faecalis* to *E. coli* depends on the mating potential of the donor bacteria.

Conjugative transposition of Tn*916* from *E. faecalis* to *E. coli* by *trans* activation. The results presented above suggest that the apparent inability of Tn*916* to transfer from *E. faecalis* to *E. coli* and *P. fluorescens* is due to the fact that *tet*(*M*), when part of the chromosome of these hosts, does not allow selection of tetracycline resistance transconjugants. However, failure to detect conjugal transfer of Tn*916* from *E. faecalis* to these gram-negative recipients may reflect the fact that EFCP1 was a less efficient transposon delivery donor than EFCP2 (Table 2). To discriminate between these two non-mutually exclusive hypotheses, Tn*916* was introduced by conjugation into EFCP2 to give EFCP7 (Table 1). In crosses between *E. faecalis* EFCP7 and BM4110, transfer of Tn*916* and that of Tn*916*-Km occurred at similar frequencies of approximately 10^{-4} per donor (Table 3). Fifty percent of the transconjugants selected on media containing kanamycin were resistant to tet-

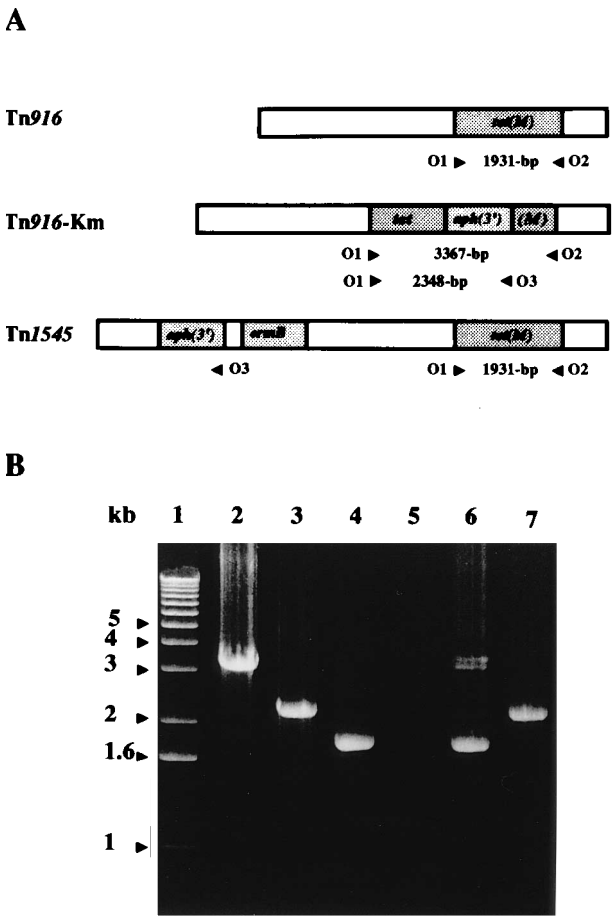

FIG. 3. Analysis of the transposon content of *E. coli* transconjugants by PCR. (A) Schematic representation of transposons Tn*916*, Tn*916*-Km, and Tn*1545*. *aph*(*3′*), gene encoding 3′-aminoglycoside phosphotransferase [APH(3′)-III]; *ermB*, gene encoding rRNA methylase (ERMB), and *tet*(*M*), gene encoding TET(M) protein. The positions of the PCR primers O1, O2, and O3 in *tet*(*M*) and in *aph*(*3′*) are indicated. (B) Amplification by PCR of bacterial DNAs from *E. coli* TB1 transconjugants harboring Tn*916*-Km (lanes 2 and 3), Tn*916* or Tn*1545* (lanes 4 and 5), or Tn*916*-Km plus Tn*916* or Tn*1545* (lanes 6 and 7). Lanes 2 to 7, 10 μl of the PCR mixtures obtained with the primer pair O1-O2 (lanes 2, 4, and 6) or O1-O3 (lanes 3, 5, and 7); lane 1, 1-kb ladder.

racycline, indicating that they had received Tn*916*-Km and Tn*916* (Table 3). We assumed that, in EFCP7, transfer of Tn*916* was activated by Tn*916*-Km (10). When EFCP7 donors were mated with TB1 recipients, we obtained *E. coli* transconjugants on selective media containing kanamycin but not on media containing tetracycline (Table 3). Chromosomal DNA was prepared from all *E. coli* transconjugants resistant to kanamycin and tested for the presence of Tn*916* by PCR. The primers O1 (5′-TTAGCTCATGTTGATGCGGGA-3′) and O2 (5′-TGGCAAACAGGTTCACCGGTA-3′) were used to amplify a 1,931-bp DNA fragment specific for Tn*916* (Fig. 3). This analysis revealed that forty percent of *E. coli* transconjugants selected on kanamycin had also received Tn*916*, although they remained susceptible to tetracycline (data not shown). These results demonstrate that conjugal transfer of Tn*916* from *E. faecalis* to the *E. coli* chromosome cannot give rise to selectable tetracycline-resistant transconjugants.

Conjugative transposition of Tn*1545* from *E. faecalis* to *E. coli* by *trans* activation. Tn*1545* is an element closely related to Tn*916*, which carries the *aph-3′* and *ermB* resistance genes in addition to *tet*(*M*) (9). This naturally occurring transposon should therefore be able to carry out conjugative transposition from *E. faecalis* to *E. coli*. However, in agreement with results obtained by others (9), all attempts to transfer Tn*1545* from *E. faecalis* EFCP4 to *E. coli* TB1 were unsuccessful (Table 2). In crosses between *E. faecalis* strains, EFCP4 was a less efficient transposon delivery donor than EFCP2 or EFCP7. Therefore, although carefully selected, EFCP4 may not possess a sufficiently high donor potential to enable conjugal transfer of Tn*1545* in heterogeneric matings between *E. faecalis* and *E. coli*. To test this hypothesis, Tn*1545* was introduced by conjugation into EFCP1 and EFCP2 to give EFCP5 and EFCP6, respectively (Table 1). We investigated whether, in these *E. faecalis* donors, transfer of Tn*1545* was activated by Tn*916* or Tn*916*-Km. In crosses between *E. faecalis* EFCP5 and *E. faecalis* JH2-2, transfer of *tet*(*M*) was obtained with an average frequency of 7×10^{-6}. Seventy percent of these tetracycline-resistant transconjugants were susceptible to kanamycin and erythromycin, i.e., the resistance markers of Tn*1545*, and therefore had received Tn*916* only (Table 4). Thus, EFCP5 delivered Tn*916* and Tn*1545* at similar frequencies of approximately 5×10^{-6} per donor, i.e., this strain delivered Tn*916* less efficiently than EFCP1 but delivered Tn*1545* at a frequency similar to that for EFCP4 (Tables 2 and 4). In matings between *E. faecalis* strains, the donor efficiency of EFCP6 was 1 order of magnitude lower than that of EFCP2 (Tables 2 and 4). Consequently, EFCP6 delivered Tn*916*-Km less efficiently than did EFCP2 but delivered Tn*1545* at a frequency 1 order of magnitude higher than that obtained with EFCP4 as the donor. We interpret these results as indicating that, in EFCP5 and EFCP6, Tn*1545* exerted an inhibitory effect on the transfer of Tn*916* and of Tn*916*-Km, respectively. In crosses between EFCP5 and *E. coli* TB1, transfer of Tn*916* and/or Tn*1545* was never detected (Table 4). In contrast, when EFCP6 donors were mated with TB1 recipients, we obtained *E. coli* transconjugants on selective media containing kanamycin or erythromycin (Table 4). These results suggest that, in our experimental conditions, conjugative transposons that transfer at frequencies equal or superior to 4×10^{-5} between *E. faecalis* strains can carry out conjugative transposition from *E. faecalis* to *E. coli*. Transconjugants of *E. coli* harboring Tn*1545*, i.e., those resistant to erythromycin and kanamycin, were susceptible to tetracycline (data not shown). This observation is consistent with the fact that we never obtained *E. coli* transconjugants harboring Tn*1545* on media containing tetracycline (Table 4). The presence of Tn*1545* alone or associated with Tn*916*-Km was tested by PCR on chromosomal DNAs from all *E. coli* erythromycin- and kanamycin-resistant transconjugants. Primers O1 and O2 were used to amplify 1,931- and 3,367-bp DNA fragments from Tn*1545* and Tn*916*-Km, respectively (Fig. 3). We also used primers O1 plus O3 (5′-TGCTTTTTA GACATCTAAATCTAGG-3′), which amplify a 2,348-bp DNA fragment specific to Tn*916*-Km (Fig. 3). This analysis revealed that 10% of *E. coli* transconjugants selected on kanamycin and 25% of those selected on erythromycin had received Tn*1545* only. Southern analysis showed that Tn*1545* was transferred from *E. faecalis* to *E. coli* without detectable rearrangement of its structure (data not shown). Thus, conjugative transposition of Tn*1545* from *E. faecalis* to *E. coli* by *trans* activation with Tn*916*-Km can occur without coestablishment of the activating transposon in the recipient.

In conclusion, our study demonstrates that transposons belonging to the Tn*916* family can carry out conjugative transposition from the chromosome of *E. faecalis* to those of *E. coli* and *P. fluorescens* and that this event depends largely on the donor potential of the *E. faecalis* transposon delivery strain. Since the *tet*(*M*) gene does not confer selectable tetracycline

TABLE 4. Conjugative transposition of Tn*1545* from *E. faecalis* to *E. coli* by *trans* activation

E. faecalis donor[a]	Recipient	Antibiotic selection[b]	No. of transconjugants/ donor (avg ± SD)[c]	Transposon transfer selection	% Transconjugants with:	
					No Tn*1545*[d]	Only Tn*1545*[e]
EFCP5	*E. faecalis* JH2-2	RIF, FUS, TC	$(6.9 \pm 1.2) \times 10^{-6}$	Tn*916*, Tn*1545*	70	ND
EFCP5	*E. faecalis* JH2-2	RIF, FUS, KM	$(3.5 \pm 1.7) \times 10^{-6}$	Tn*1545*	0	ND
EFCP5	*E. coli* TB1	VAN, TC	$<10^{-9}$	Tn*916*, Tn*1545*		
EFCP5	*E. coli* TB1	VAN, KM	$<10^{-9}$	Tn*1545*		
EFCP6	*E. faecalis* BM4110	SM, KM	$(4.6 \pm 1.8) \times 10^{-5}$	Tn*916*-Km, Tn*1545*	60	ND
EFCP6	*E. faecalis* BM4110	SM, TC	$(2.4 \pm 1) \times 10^{-5}$	Tn*1545*	0	ND
EFCP6	*E. coli* TB1	VAN, KM	$(3.2 \pm 1.7) \times 10^{-9}$	Tn*916*-Km, Tn*1545*	75	10
EFCP6	*E. coli* TB1	VAN, EM	$(2.5 \pm 1.5) \times 10^{-9}$	Tn*1545*	0	25
EFCP6	*E. coli* TB1	VAN, TC	$<10^{-9}$	Tn*1545*		

[a] EFCP5, BM4110::Tn*916*::Tn*1545*; EFCP6, JH2-2::Tn*916*-Km::Tn*1545*.
[b] Antibiotics were used at the concentrations indicated in Table 2, footnote *b*. RIF, rifampin; FUS, fusidic acid; TC, tetracycline; KM, kanamycin; VAN, vancomycin; SM, streptomycin; EM, erythromycin.
[c] The data are means for three independent matings.
[d] Transconjugants susceptible to erythromycin.
[e] Determined by PCR (Fig. 3). ND, not determined.

resistance to certain gram-negative bacteria including *E. coli*, the presence of additional markers readily expressed in these recipients (for example, those carried by Tn*1545*) is required for the detection of this type of transfer. Therefore, as previously suggested (28), reports of conjugal transfer of wild-type Tn*916* from *E. faecalis* to *E. coli*, *A. eutrophus*, and *C. freundii* (3), in which it conferred resistance to high levels (≥10 μg/ml) of tetracycline, remain questionable. Finally, our findings better define the limits of promiscuity of these broad-host-range conjugative transposons and, combined with a previous report (33), lend further support for the hypothesis that enterococci and streptococci can serve as a reservoir of (resistance) genes for gram-negative bacteria.

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ADDENDUM IN PROOF

Since this paper was accepted for publication, the nucleotide sequence of Tn*916* has been made available in the GenBank database. Our finding that conjugal transfer of Tn*916*-Km is not sensitive to restriction by *Eco*K is consistent with the fact that there is no cleavage site for this enzyme in this element.

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